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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: **John Shanklin**

Serial No.: **10/017,145** Group No. **1652**

Filed: **December 14, 2001** Examiner: **T. Saidha**

Title: **MUTANT FATTY ACID DESATURASE**

DECLARATION UNDER 37 C.F.R. §1.132

The Honorable Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, John Shanklin, declare and say:

1. I received a B.Sc. in Biology from the University of Lancaster, England in 1981 and an M.Sc. in 1984 and a Ph.D. in 1988 in Forestry and Horticulture from the University of Wisconsin, Madison.

2. I was a postdoctoral fellow at the U.S. Department of Energy (US DOE) Plant Research Laboratory at Michigan State University, East Lansing, MI from 1988 to 1992.

3. I have been on the scientific staff of Brookhaven National Laboratory (BNL) since 1992. I am currently a Senior Biochemist of the Biology Department at BNL and an adjunct professor at the State University of New York at Stony Brook.

4. I have published 58 peer-reviewed papers in the scientific literature. Much of my research since my postdoctoral studies has been directed toward the development of an

understanding of the structure-function relationships that control enzyme substrate and reactivity specificity. These efforts have primarily been directed toward the understanding of these relationships in enzymes involved in lipid metabolism, and particularly in di-iron desaturases. I have published approximately 40 papers and reviews related to this topic in the scientific literature.

5. I am an inventor of the above-referenced patent application.

6. **Guidance of Reference 1: Cahoon et al., (1997) PNAS 94:4872-4877.**

After a thorough review of the following references:

1. Cahoon et al., [PNAS 94:4872-4877, May 1997];

2. US Patent 5,705,391 (Cahoon et al, Jan 6, 1998); and

3. US Patent 5,888,790 (Cahoon et al, March 30, 1999),

I found that Figure 4 of Reference 1 presents a logic-based way <u>to begin</u> to alter the activity of a Δ^9-18:0 desaturase to cause an increase in activity toward fatty acids having fewer than 18 carbon atoms.

This logic-based method of determination of which amino acid residues are relevant to a particular aspect of the catalytic function of an enzyme involves aligning amino acid sequences of enyzmes that carry out an identical catalytic activity but do so specifically on different substrates. Thus, Figure 4 in Reference 1, which compares homologous residues located along the binding channel of 4 different acyl-ACP desaturases (actually 5 are compared since the Δ^9-18:0 desaturases from castor and *T. alata* are both shown), <u>**may**</u> provide guidance that could be used as a <u>**starting point**</u> for creating a mutant castor Δ^9-18:0-ACP desaturase which has an increased activity toward fatty acid substrates having fewer than 18 carbon atoms.

The Figure shows the following:

Table 1

Residue number of castor Δ^9-18:0-ACP desaturase	Amino Acid in castor Δ^9-18:0-ACP desaturase (*T. alata* Δ^9 desaturase)	Homologous residue in *T. alata* Δ^6 **16:0** desaturase[a,b]	Homologous residue in geranium Δ^9 **14:0** desaturase[a,b]	Homologous residue in coriander Δ^4 **16:0** desaturase[a,b]
114	Met (Met)	*Met*	**Leu**	*Met*
115	Leu (**Ile**)	*Leu*	**Val**	*Leu*
117	Thr (Thr)	*Thr*	**Arg**	**Arg**
118	Leu (Leu)	**Thr**	**Pro**	**Cys**
179	Pro (Pro)	*Pro*	**Ile**	**Thr**
181	Thr (Thr)	**Ala**	*Thr*	*Thr*
188	Gly (Gly)	**Ala**	**Leu**	*Gly*
189	Phe (Phe)	**Tyr**	**Tyr**	*Phe*

[a]Residues differing from those of the castor Δ^9-18:0 desaturase are indicated in **bold typeface**.
[b]Residues identical to those of the castor desaturase are indicated in *italicized typeface*

Thus, based on Reference 1, Figure 4, one of skill in the art could make use of Table 1 as a starting point to consider which amino acid changes **might** create a mutant enzyme having the desired change in activity.

As a beginning, the chart suggests that:

- It would be reasonable that Met 114 remain unchanged because two of the three desaturases with specificity for shorter fatty acids also have Met in the homologous position.
- One might consider changing Thr 117 to Arg, except that the *T. alata* 16:0 desaturase has a Thr in the homologous position.
- The data for Leu 118 suggest that it could be replaced with either Thr, Pro or Cys to **possibly** create a desaturase with the desired activity.
- For Pro 179, one might consider changing it to either Ile or Thr, except that the *T. alata*

16:0 desaturase also has a Pro in the homologous position.

- It would be reasonable that Thr 181 remain unchanged since two of the three desaturases with specificity for shorter fatty acids have Thr in that position.
- For Gly 188, one might consider changing it to Ala or Leu, except that the coriander 16:0 desaturasė also has Gly in that position.

Because none of the desaturases having a specificity for fatty acids with fewer than 18 carbon atoms has Leu at position 118 and because for all of the other residues at least one of the desaturases has the same residue as that in the castor 18:0 desaturase, the most likely change that one of skill in the art **might first elect to try** would be to change Leu 118 to some other amino acid, possibly Thr, Pro or Cys.

Because only Leu 118 is different in the desaturases that are specific for the shorter chain fatty acid substrates, merely making one or more of the substitutions suggested in Table 1 would be unlikely to guarantee success in making a working mutant castor Δ^9-18:0-ACP desaturase having an increased activity towards fatty acids with fewer than 18 carbon atoms.

In fact, comparing the 12 amino acids shown in Table 1 that differ from the Δ^9-18:0 desaturases to the substitutions claimed in the present application shows that merely using the changes in Table 1, either making the substitutions one by one or in combinations (there are more than 800 possible combinations of amino acids listed in the Table for the 8 positions) would not guide one to make any of the claimed mutants of the present invention, with the possible exception of the T117R/G188L mutant.

However, because this double mutant is one of over 800 potential combinatorial choices, one would be required to undertake a considerable effort to arrive at this particular mutant. If

one began by making all possible single amino acid substitutions, one would need to make 12 mutants. If one then made all possible double mutants, 61 additional mutants would be required to arrive at the T117R/G188L double mutant.

In addition, since none of the other residue changes suggested in Figure 4 are changes claimed in the current patent application, the suggestions of Figure 4 would be absolutely unlikely to result in any of the multi-substituted mutant desaturases claimed in the present invention.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true. Further that these statements were made with the knowledge that willfully false statements, and the like, so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willfully false statements may jeopardize the validity of patent that may issue from the referenced application.

Aug 5 2003
Date

John Shanklin, Ph. D.